

(6)

(19) Japan Patent  
Office (JP)  
(12) Japanese Patent  
Laid-Open Application  
Publication (A)

(11) Patent Laid-Open  
Application  
No. Hei 3-219872

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(54) Title of the Invention: LIPASE INHIBITOR

(21) Patent Application: No. Hei 2-13699  
(22) Filing Date: January 25, 1990

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### Specification

1. Title of the Invention:

LIPASE INHIBITOR

2. Claims for the Patent:

(1) A lipase inhibitor comprising a water extract of a natural food product (excluding seed of Nerima Daikon (Japanese radish)).

(2) The lipase inhibitor according to Claim 1, wherein the natural food product is at least one selected from the group consisting of sweet pepper, pumpkin, Lyophyllum shimeji, Grifola frondosa, Hizikia fusiformis, green tea, black tea, and oolong tea.

3. Detailed Description of the Invention:

[Industrial Application Field]

The present invention relates to a lipase inhibitor, and more specifically relates to a lipase inhibitor derived from a natural food product.

[Conventional Art, Problems to be Solved by the Invention]

Fats contained in foods are hydrolyzed by microorganism lipase or the like to glycerol and free fatty acids. These free fatty acids cause decomposition of foods and a foul odor and are a significantly large problem in quality maintenance of foods.

In order to prevent such degradation of foods by lipase, in conventional methods, for example, foods are heated for inactivating the enzyme; triglyceride that is relatively lowly degraded by lipase, namely, triglyceride composed of long chain fatty acids containing no double bond, is used; or a lipase inhibitor contained in seed of Nerima Daikon (Japanese radish) is added to foods.

However, in the above first method, the inactivation of the enzyme by heating is insufficient in some foods. For example, microorganism-derived lipase contained in cacao beans is not inactivated even if the cacao beans are roasted at 150°C. In the second method, since triglyceride that can be used is limited, the food processing is restricted, and also the effect obtained is insufficient. Furthermore, in the third method, the lipase inhibitor is limited to only those contained in seed of Nerima Daikon, and also it is not confirmed whether the lipase can be applied to various foods.

[Means for Solving the Problems]

Accordingly, the present inventors have investigated for obtaining lipase inhibitors from natural food products other than seed of Nerima Daikon and, as a result, have found that water extracts of specific natural food products have activities of inhibiting lipase. The present invention has been accomplished based on this finding.

That is, the present invention provides a lipase inhibitor including a water extract of a natural food product (excluding seed of Nerima Daikon (Japanese radish)).

Examples of the natural food product used in the present invention include sweet pepper, pumpkin, *Lyophyllum shimeji*, *Grifola frondosa*, *Hizikia fusiformis*, green tea, black tea, and oolong tea.

The extraction with water from the above natural food product can be performed by various methods. For example, a suitable amount of water is added to a natural food product and mixed with pulverizing, and the mixture is then filtered to obtain filtrate. Then, the filtrate is heated at 100°C for about 10 minutes for inactivating the enzyme in the filtrate. In the present invention, water includes not only room temperature water but also hot water.

In a case that a lipase inhibitor of the present invention is used in a food, the above-mentioned aqueous solution may be directly used or may be used after pulverization by lyophilization or the like, according to necessity. Furthermore, the amount of use of the lipase inhibitor of the present invention may be properly determined with considering the lipase content in a target food. For example, a lipase inhibitor of about 5 to 30 mL per 1 U of lipase can be a guideline. Though there is animal-derived lipase in addition to microorganism-derived lipase, either of the both is effective as a lipase inhibitor of the present invention.

[Example]

Then, the present invention will be described in more detail with reference to Example.

#### Manufacturing Example

The extraction with water from the following natural food products was performed as follows. That is, water at an amount shown in Table 1 was added to 1 part by weight of each food product and mixed with a homo-mixer for five minutes. The amount of water differed depending on the food product for efficient extract by using water in an amount suitable for the extraction. After the mixing, the mixture was centrifuged at 30000 G for 10 minutes to give a supernatant. This supernatant was filtered, and the resulting aqueous solution was heated at 100°C for 10 minutes to precipitate macromolecular components. The aqueous solution was filtered again to give a water extract of the natural food product.

Table 1

<u>Natural food product</u>	<u>Water amount (part by weight)</u>
Sweet pepper	0.25
Pumpkin	0.75
Lyophyllum shimeji	0.25
Grifola frondosa	0.25
Hizikia fusiformis	0.5
Green tea	5.0
Black tea	5.0
Oolong tea	5.0

Then, the lipase inhibiting activities by the respective aforementioned water extracts were measured by the following

method. The water extract and a predetermined amount of microorganism-derived lipase were added to 4-methyl umbelliferyl oleate as a substrate, and a reaction was performed at 37°C for 20 minutes. Then, fluorescence intensity of the produced 4-methyl umbelliferone was measured. Specifically, 50 µL of the water extract and 50 µL of a microorganism-derived lipase solution (trade name: Lipase F, manufactured by Amano Pharmaceutical Co., Ltd., 128500 U/g, McIlvaine buffer solution) were added to 100 µL of a substrate solution (43.9 mg/L, pH 8.0, McIlvaine buffer solution), followed by a reaction at 37°C for 20 minutes. Then, the reaction was terminated with 3.0 mL of a borate buffer solution with a pH of 10.0. The fluorescence intensity was measured at an excitation wavelength of 360 nm and a fluorescence wavelength of 450 nm. The lipase activity inhibition ratio was determined by the expression shown below. Table 2 shows the results. Similarly, the water extract was also measured for an activity inhibiting an animal-derived lipase by using porcine pancreatic lipase (manufactured by Wako Pure Chemical Industries, 676 U/g). The results are shown in Table 2.

Lipase activity inhibition ratio =

$$[1-(A-a)/(B-b)] \times 100$$

A: fluorescence intensity when a solution under test was added,

a: fluorescence intensity of a blank solution of the above,

B: fluorescence intensity when water was added, and

b: fluorescence intensity of a blank solution of the above.

Table 2

<u>Natural food product</u>	<u>Microorganism lipase inhibition ratio (%)</u>	<u>Porcine pancreatic lipase inhibition ratio (%)</u>
Sweet pepper	92.6	87.3
Pumpkin	88.5	100.0
Lyophyllum shimeji	97.2	97.4
Grifola frondosa	100.0	100.0
Hizikia fusiformis	98.3	94.4
Green tea	100.0	100.0
Black tea	98.0	100.0
Oolong tea	99.0	100.0

As clear from the Table, all of the extracts of the natural food products shown in the Table have excellent lipase inhibition activities.

[Advantages of the Invention]

Every water extracts of the natural food products according to the present invention has an excellent lipase inhibition activity. Therefore, these water extracts are useful for preventing a problem in food processing, i.e., degradation caused by lipase.